Genes for diagnosing colorectal cancer

Reference cited

1. WO0055351

Field of the invention

This invention relates to genes for diagnosing colorectal cancer, particularly provided a method of clinical diagnosis for colorectal cancer which enables the effects of early diagnosis, specificity, highly sensitivity and safety.

Background of the invention

Colorectal cancer is one of the most common malignant tumors of the world, it is the second most frequent cause of malignant tumor related mortality in developed countries. In developed countries, mortality rate caused by colorectal cancer seems have a descending tendency progressively in previous 20 years, the main causes for early diagnosis is provided and the improvement of methods of therapy and medicines. But in Taiwan the reason of changing in diet habit is occidental habit input and the rapid changing in

environment, the rate of suffering for colorectal cancer is rising constantly, furthermore, also showing an age-descending tendency.

According to top ten related cancer of Taiwanese of 2002, colorectal cancer (CRC) is the third leading cause of cancer-related death for male and female, which is announced by The Department of Health (DOH), highest level of the executive branch, Taiwan. About 6681 new cases of colorectal cancer diagnosed per year such as statistical data by DOH of 1999, for 3649 patients dead in the colorectal cancer per year such as statistical data by DOH of 2002 In Taiwan. The average age of colorectal cancer patient is lower than other countries. In other words, twenty-year-old or thirty-year-old people suffer from the colorectal cancer in Taiwan. Therefore, we can't ignore the possibility of the colorectal cancer caused by young person.

Although methods of diagnosis and surgical operation treatment are improved for colorectal cancer patients, if make a comparison between early diagnosis with later period diagnosis by surgical operation respectively, the treatment is able to probably overcome the colorectal cancer in early diagnosis, but is not able to absolutely overcome the colorectal cancer in the later period diagnosis. The far metastasis are main problem of the treatment for the colorectal cancer, therefore, if a method with highly sensitivity, highly

specificity and easily diagnosis which is able to detect early and potentially curable CRC, We believe that is a novel target for CRC diagnosis and therapy.

The present invention is to provide functional genetic method, for diagnostic genes of colorectal cancer consist of 71 types of genes, that can be applied for early diagnosing possibility of recurrence and metastasis for colorectal patients. Simultaneously, tracing 100 colorectal cancer cases, found that 92% genes variation in colorectal tissue. In the process of tracing for 100 colorectal cancer cases simultaneously, mutation of genes is found in 92% colorectal cancer tissues. In the tracing process, although CEA of 16 patients still in normal value range, that detect early tumor cells in blood by using genes variation testing.

In WO0055351, ROSEN CRAIG A et. al., "Human Colon Cancer Associated Gene Sequences And Polypeptides", disclose colon cancer related polynucleotides and the polypeptides encoded by the polynucleotides herein collectively known as "colon cancer antigens", screening methods for identifying agonists and antagonists of colon cancer antigens of the invention, But, the present invention is to provide SSH and cDNA microarray technology to identify candidate marker genes which are overexpressed

continuously from colorectal proliferous polypus to colorectal oncogene, detecting overexpressed genes are selected from up regulation genes which related intently in colorectal cancer oncogene, and down regulation genes which related in colorectal cancer oncogene. The total 71 genes are used to diagnosing early colorectal cancer.

Summary of the invention

Therefore, the main purpose according to this present invention is to provide the methods of clinical diagnosis for colorectal cancer for early diagnosis, specificity, highly sensitivity and safety.

For the purpose stated above, the gene sequences comprise the steps of: (1) deriving epithelium cells from normal intestines, polypus of intestines and colorectal cancer tissue; (2) collecting genes with highly differential gene expression by Suppression Subtractive Hybridization (SSH), and building library ; (3) deriving colonies with relatively high signal intensities from cancer tissue; (4) collecting more clinically cancer tissues by Northern Hybridization, real-time Polymerase Chain Reaction (PCR) combined with analysis of bioinformation to affirm variation between differential gene expression; and (5) selecting the most suitable genes from said library. Moreover, the reagent

uses the gene sequence as method of clinical diagnosis for colorectal cancer to the early diagnosis.

Brief description of the drawings

The present invention will be better understood from the following detailed description of preferred embodiments of the invention, taken in conjunction with the accompanying drawings, in which

Table 1 is a table showing the result of clinical examination of colorectal cancer biochip;

FIG. 1 is a view showing the procedure of deriving genes according to the present invention;

FIG. 2a and FIG. 2b are views showing the primary screening according to the present invention;

FIG. 3a and FIG.3b are views showing affirmation to genes using Northern Blotting method according to the present invention;

FIG.4a and 4b are views showing quantity expression of cancer tissue according to the present invention; and

FIG.5 is a diagram showing second preferred embodiments according to the present invention.

Description of the preferred embodiments

The following descriptions of the preferred embodiments are provided to understand the methods and the procedures of the present invention. Please refer to FIG.1, showing the procedure of searching genes according to the present invention. Said procedure comprise the steps of: (1) deriving epithelium cells from normal intestines, polypus of intestines and colorectal cancer tissue; (2) collecting genes with highly differential gene expression by Suppression Subtractive Hybridization (SSH), and building library ; (3) deriving colonies with relatively high signal intensities from cancer tissue; (4) collecting more clinically cancer tissues by Northern Hybridization, real-time Polymerase Chain Reaction (PCR) combined with analysis of bioinformation to affirm variation between differential gene expression; and (5) selecting the most suitable genes from said library. Moreover, by using the gene sequence as a reagent, this enables clinical diagnosis for colorectal cancer to the effects of early diagnosis, specificity, highly sensitivity and safety.

The genes for diagnosing colorectal cancer, the specific oligonucleotides sequence are selected from the group consisting of:

No	Hs ID	Acc No	Discription	Definition	Oligo sequence
1	Hs.107213	BC027178 (SEQUENCE LISTING 72)	FNBP3 Formin binding protein 3	Homo sapiens, formin binding protein 3, clone MGC:16979 IMAGE:4343048, mRNA, complete cds	CATCATAGGAA ACGTTCCCGCT CTCGATCGGGG TCAGATTCAGAT GATGATG (SEQUENCE LISTING 1)
2	Hs.123107	NM_002257 (SEQUENCE LISTING 73)	KLK1 Kallikrein 1, renal/pancreas/salivary	Homo sapiens kallikrein 1, renal/pancreas/salivary (KLK1), mRNA.	GCCTTCTGTCG CCGTCAGAGTG CTGTCTTATGTG AAGTGGATCGA GGACA (SEQUENCE LISTING 2)
3	Hs.1369	NM_000574 (SEQUENCE LISTING 74)	DAF Decay accelerating factor for complement (CD55, Cromer blood	Homo sapiens decay accelerating factor for complement (CD55, Cromer	GGGCAGTCAAT GGTCAGATATT GAAGAGTTCTG CAATCGTAGCT GCGAGGTG (SEQUENCE

			group system)	blood group system) (DAF), mRNA	LISTING 3)
4	Hs.151254	NM_005046 (SEQUENCE LISTING 75)	KLK7 Kallikrein 7 (chymotryptic, stratum corneum)	Homo sapiens kallikrein 7 (chymotryptic, stratum corneum) (KLK7), transcript variant 1, mRNA.	TGGAACCACCT GTACTGTCTCC GGCTGGGGCAC TACCACGA (SEQUENCE LISTING 4)
5	Hs.1526	NM_001681 (SEQUENCE LISTING 76)	ATP2A2 ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	Homo sapiens ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 (ATP2A2), mRNA	CATCGGCATCT TCGGGCAGGAT GAGGACGTGAC GTCAAAAGCTTT CACAG (SEQUENCE LISTING 5)
6	Hs.184270	NM_006135 (SEQUENCE LISTING 77)	CAPZA1 Capping protein (actin	Homo sapiens capping protein (actin filament)	TGACCACTTAC GGAAAGAAGCA AGTGACCCCCA

			filament) muscle Z-line, alpha 1	muscle Z-line, alpha 1 (CAPZA1), mRNA.	GCCAGAAGAAG CAGATG (SEQUENCE LISTING 6)
7	Hs.2043	NM_001151 (SEQUENCE LISTING 78)	SLC25A4 Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Homo sapiens solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4 (SLC25A4), nuclear gene encoding mitochondrial protein, mRNA.	AGATCTTCAAGT CTGATGGCCTG AGGGGGCTCTA CCAGGGTTTCA ACGTC (SEQUENCE LISTING 7)
8	Hs.267871	NM_005177 (SEQUENCE LISTING 79)	ATP6V0A1 ATPase, H+ transporting, lysosomal V0	Homo sapiens ATPase, H+ transporting, lysosomal V0	GGACAGAAAGG AATTCAGTGTTT CCTGGTAGTGG TTGCACTACTGT

			subunit a isoform 1	subunit a isoform 1 (ATP6V0A1), mRNA.	GTGTACCTTGG (SEQUENCE LISTING 8)
9	Hs.4935	D79998 (SEQUENCE LISTING 80)	KIAA0176 KIAA0176 protein	Human mRNA for KIAA0176 gene, partial cds	GGAAAGGATAC GGGACAATGAG AACAGAACTTCA CAAGGCCCCGT GAAGC (SEQUENCE LISTING 9)
10	Hs.5509	NM_006495 (SEQUENCE LISTING 81)	EVI2B Ecotropic viral integration site 2B	Homo sapiens ecotropic viral integration site 2B (EVI2B), mRNA.	GCCCCTGCCAC CAGTAGATTTTA TGAAAAACCAA GAAGATTCCAA CCTTGAGATCC AGTGTC (SEQUENCE LISTING 10)
11	Hs.5662	NM_006098 (SEQUENCE	GNB2L1 Guanine	Homo sapiens guanine	ATGACTGAGCA GATGACCCTTC

		LISTING 82)	nucleotide binding protein (G protein), beta polypeptide 2-like 1	nucleotide binding protein (G protein), beta polypeptide 2-like 1 (GNB2L1), mRNA.	GTGGCACCCTC AAGGGCCACAA C (SEQUENCE LISTING 11)
12	Hs.75990	NM_005143 (SEQUENCE LISTING 83)	HP Haptoglobin	Homo sapiens haptoglobin (HP), mRNA.	AGGCTGTTGGA GATAAACTTCCT GAATGTGAAGC AGATGACGGCT GCCCG (SEQUENCE LISTING 12)
13	Hs.83384	NM_006272 (SEQUENCE LISTING 84)	S100B S100 calcium binding protein, beta (neural)	Homo sapiens S100 calcium binding protein, beta (neural) (S100B), mRNA	CCGAACTCAAG GAGCTCATCAA CAATGAGCTTTC CCATTTCTTAGA GGAAATCAAAG AGCAGGAG (SEQUENCE LISTING 13)

14	Hs.10029	NM_001814 (SEQUENCE LISTING 85)	CTSC Cathepsin C	Homo sapiens cathepsin C (CTSC), mRNA	CACCGGAAAGA AGGTGGGAACT GCCTCTGAGAA TGTGTATGTCAA CACAGC (SEQUENCE LISTING 14)
15	Hs.103982	NM_005409 (SEQUENCE LISTING 86)	SCYB11 Small inducible cytokine subfamily B (Cys-X-Cys), member 11	Homo sapiens small inducible cytokine subfamily B (Cys-X-Cys), member 11 (SCYB11), mRNA.	GGGCATGGCTA TAGCCTTGGCT GTGATATTGTGT GCTACAGTTGTT CAAGGC (SEQUENCE LISTING 15)
16	Hs.12314	AL049397 (SEQUENCE LISTING 87)	Homo sapiens mRNA; cDNA DKFZp586C1019 (from clone	Homo sapiens mRNA; cDNA DKFZp586C1019 (from clone DKFZp586C1019)	CAACACCACAG ACAGCTGCAGG ACTCGATATCCA TGGCTTCTTTCC ATCAC (SEQUENCE

			DKFZp586C1019)		LISTING 16)
17	Hs.150557	NM_001206 (SEQUENCE LISTING 88)	BTEB1 Basic transcription element binding protein 1	Homo sapiens basic transcription element binding protein 1 (BTEB1), mRNA.	TTCCACCCCAG CATGATCAAGC GATCGAAAAAG GCGCTGGCCAA CGCTTT (SEQUENCE LISTING 17)
18	Hs.169266	NM_000909 (SEQUENCE LISTING 89)	NPY1R Neuropeptide Y receptor Y1	Homo sapiens neuropeptide Y receptor Y1 (NPY1R), mRNA.	CCGGTCTCGGG ATGATGATTATG AAACAATAGCC ATGTCCACGAT GCACACAG (SEQUENCE LISTING 18)
19	Hs.1827	NM_002507 (SEQUENCE LISTING 90)	NGFR Nerve growth factor receptor (TNFR superfamily,	Homo sapiens nerve growth factor receptor (TNFR superfamily,	CAAGCGGGAGG AGGTGGAGAAG CTTCTCAACGG CTCTGCG (SEQUENCE

			member 16)	member 16)	LISTING 19)
20	Hs.1869	NM_002633 (SEQUENCE LISTING 91)	PGM1 Phosphoglucomutase 1	(NGFR), mRNA. Homo sapiens phosphoglucomutase 1 (PGM1), mRNA.	GCCAACGGGAT CGGTCGCTTGG TTATCGGACAG AATGGAATCCT CTCCA (SEQUENCE LISTING 20)
21	Hs.194148	NM_005433 (SEQUENCE LISTING 92)	YES1 V-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	Homo sapiens v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1(YES1), mRNA	CAAGTGTGAGC CATTATGGAGC AGAACCCACTA CAGTGTCACCA TGTCCG (SEQUENCE LISTING 21)
22	Hs.2352	X74210 (SEQUENCE LISTING 93)	ADCY2 Adenylate cyclase 2 (brain)	H.sapiens mRNA for adenylyl cyclase	TCGTCTGCTTTG CTGGACAGCTT CTGCAATGCAG CAAAAAAGCCT CTCCC

					(SEQUENCE LISTING 22)
23	Hs.246885	NM_017958 (SEQUENCE LISTING 94)	FLJ20783 Hypothetical protein FLJ20783	Homo sapiens hypothetical protein FLJ20783 (FLJ20783), mRNA.	CCAAGATTCTA GGACAAACACA GCGTATGTGGG CTCTGCAGTCA TGACCG (SEQUENCE LISTING 23)
24	Hs.29665	NM_014944 (SEQUENCE LISTING 95)	CLSTN1 Calsyntenin 1	Homo sapiens calsyntenin 1 (CLSTN1), mRNA.	CACGAGCCCTT CTCTGTGACTG AGGATTACCCG CTCCATCCATC CAAGAT (SEQUENCE LISTING 24)
25	Hs.3235	NM_002272 (SEQUENCE LISTING 96)	KRT4 Keratin 4	Homo sapiens keratin 4 (KRT4), mRNA	TTCAGCTGTGG CTCGGCCATTG TAGGCGGTGGC AAGAGAGGT (SEQUENCE

					LISTING 25)
26	Hs.55209	AF327354 (SEQUENCE LISTING 97)	Homo sapiens DMR protein mRNA, complete cds	Homo sapiens DMR protein mRNA, complete cds	TAAAGTGGGCT CATTGTCATCCC CAAGCCAGGCC AGTTCTCCAGG TGGAA (SEQUENCE LISTING 26)
27	Hs.585	NM_000384 (SEQUENCE LISTING 98)	APOB Apolipoprotein B (including Ag(x) antigen)	Homo sapiens apolipoprotein B (including Ag(x) antigen) (APOB), mRNA	GCCCAAGGCCA CAGGGGTCCTT TATGATTATGTC AACAAGTACCA CTGGG (SEQUENCE LISTING 27)
28	Hs.62187	AF022913 (SEQUENCE LISTING 99)	PIGK Phosphatidylinositol glycan, class K	Homo sapiens GPI transamidase mRNA, complete cds	TCTTGTCCTTCG GCAGCGTGGCC GCTAGTCATATC GAGGATCAAGC AGAA (SEQUENCE

					LISTING 28)
29	Hs.63290	NM_012260 (SEQUENCE LISTING 100)	HPCL2 2-hydroxyphytanoyl-CoA lyase	Homo sapiens 2-hydroxyphytanoyl-CoA lyase (HPCL2), mRNA	CATGAACTGCT GGCCCTTGCTT GTGATTGGTGG TTCCTCTGAAAG AAACCAAG (SEQUENCE LISTING 29)
30	Hs.699	NM_000942 (SEQUENCE LISTING 101)	PPIB Peptidylprolyl isomerase B (cyclophilin B)	Homo sapiens peptidylprolyl isomerase B (cyclophilin B) (PPIB), mRNA	AGCCGGGATAA ACCCCTGAAGG ATGTGATCATC GCAGACTGCGG CAAGAT (SEQUENCE LISTING 30)
31	Hs.74111	NM_007367 (SEQUENCE LISTING 102)	RALY RNA binding protein (autoantigenic, hnRNP-associated	Homo sapiens RNA binding protein (autoantigenic, hnRNP-associated with	AGCGAGGAAGA GCTGGAACACA GCCAGGACACA GACGCGGATGA T (SEQUENCE

			with lethal yellow)	lethal yellow) (RALY) transcript variant 2, mRNA	LISTING 31)
32	Hs.75103	NM_003406 (SEQUENCE LISTING 103)	YWHAZ Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	Homo sapiens tyrosine 3-monooxygenase /tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ), mRNA	CGGAAGGTGCT GAGAAAAAACA GCAGATGGCTC GAGAATACAGA GAGAAAATTGA GACGG (SEQUENCE LISTING 32)
33	Hs.75117	NM_004515 (SEQUENCE LISTING 104)	ILF2 Interleukin enhancer binding factor 2, 45kD	Homo sapiens interleukin enhancer binding factor 2, 45kD (ILF2), mRNA	TGACTTCTATTT GTGTGAAATGG CCTTTCCCCGG GTCAAGCCAGC ACCTG (SEQUENCE LISTING 33)

34	Hs.75236	NM_021952 (SEQUENCE LISTING 105)	ELAVL4 ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)	Homo sapiens ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D) (ELAVL4), mRNA	GCACCATGGAG CCTCAGGTGTC AAATGGTCCGA CATCCAATACAA GCAATG (SEQUENCE LISTING 34)
35	Hs.75258	NM_004893 (SEQUENCE LISTING 106)	H2AFY H2A histone family, member Y	Homo sapiens H2A histone family, member Y (H2AFY), transcript variant 2, mRNA	CACCGAAGCCA GGAAGCCCCGT TTGTAAGCGTG TGTTGTGGTGC TTTATT (SEQUENCE LISTING 35)
36	Hs.75498	NM_004591 (SEQUENCE LISTING 107)	SCYA20 Small inducible cytokine subfamily A	Homo sapiens small inducible cytokine subfamily A (Cys-Cys),	GCTACTCCACC TCTGCGGCGAA TCAGAAGCAGC AAGCAACTTTGA CTGCT

			(Cys-Cys), member 20	member 20 (SCYA20), mRNA	(SEQUENCE LISTING 36)
37	Hs.76913	NM_002790 (SEQUENCE LISTING 108)	PSMA5 Proteasome (prosome, macropain) subunit, alpha type, 5	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 5 (PSMA5), mRNA	GTTTCTTACCCG GTCTGAGTACG ACAGGGGCGTG AATACTTTTTCT CCCG (SEQUENCE LISTING 37)
38	Hs.79889	NM_012329 (SEQUENCE LISTING 109)	MMD Monocyte to macrophage differentiation-associated	Homo sapiens monocyte to macrophage differentiation-associated (MMD), mRNA	GCTATGAACAT GCTGCTAACTG TTACACACACG CATTCCTCATTG TTCCGGCC (SEQUENCE LISTING 38)
39	Hs.82173	NM_005655 (SEQUENCE LISTING 110)	TIEG TGFB inducible early growth	Homo sapiens TGFB inducible early growth	TTTGTGGTACC CCAGCCCGTTG TGCAGAGTTCA

			response	response (TIEG), Mrna	AAGCCTCCGGTG (SEQUENCE LISTING 39)
40	Hs.84072	NM_004616 (SEQUENCE LISTING 111)	TM4SF3 Transmembrane 4 superfamily member 3	Homo sapiens transmembrane 4 superfamily member 3 (TM4SF3), mRNA	GCAATGACTCTCAAGCAATTTTTGGTTCTGAAGATGTAGGCTCTAGCTCCTACGTTGCTGTG (SEQUENCE LISTING 40)
41	Hs.85146	NM_005239 (SEQUENCE LISTING 112)	ETS2 V-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	Homo sapiens v-ets erythroblastosis virus E26 oncogene homolog 2 (avian) (ETS2), mRNA	CTCATGACTCCGCCAACTGTGAATTGCCTTTGTTAACCCCGTGCAGCAAG (SEQUENCE LISTING 41)
42	Hs.85844	NM_002529	NTRK1	Homo sapiens	TTCATGGACAA

		(SEQUENCE LISTING 113)	Neurotrophic tyrosine kinase, receptor, type 1	neurotrophic tyrosine kinase, receptor, type 1 (NTRK1), mRNA	CCCTTTCGAGTT CAACCCCGAGG ACCCCATCCCT GTCT (SEQUENCE LISTING 42)
43	Hs.88219	NM_003454 (SEQUENCE LISTING 114)	ZNF200 Zinc finger protein 200	Homo sapiens zinc finger protein 200 (ZNF200), mRNA	CCCAGTCAGAA AGTCAAGGAGA CCTTGGTTATTA TGAAAGATGTG AGCTCAAGCCT TCAGAACAG (SEQUENCE LISTING 43)
44	Hs.9914	NM_006350 (SEQUENCE LISTING 115)	FST Follistatin	Homo sapiens follistatin (FST), transcript variant FST317, mRNA	CCCTGACAGTA AGTCGGATGAG CCTGTCTGTGC CAGTGACAATG CCACTT (SEQUENCE LISTING 44)

45	Hs.169319	NM_003419 (SEQUENCE LISTING 116)	ZNF345 Zinc finger protein 345	Homo sapiens zinc finger protein 345 (ZNF345), mRNA	CAGGGATCTCA GGAAGGACATT TCAGTGAAATG ATATTTACTCCT GAAGACATGCC CACTTTCAG (SEQUENCE LISTING 45)
46	Hs.72805	NM_030921 (SEQUENCE LISTING 117)	DC42 Hypothetical protein DC42	Homo sapiens hypothetical protein DC42 (DC42), mRNA	GGCATGGCAGC AAATGCCAACAT TTTGTGGAATAG CAGCAAATCTA CAAGAGACCCT GG (SEQUENCE LISTING 46)
47	Hs.108301	NM_003297 (SEQUENCE LISTING 118)	NR2C1 Nuclear receptor subfamily 2, group C,	Homo sapiens nuclear receptor subfamily 2, group C, member 1	GACACCTACAG GTTATCCAGACT ACTACTCAGATT GCCAGCTTTAA GACTGATGAAT

			member 1	(NR2C1), mRNA	GCTACCATC (SEQUENCE LISTING 47)
48	Hs.177926	NM_030941 (SEQUENCE LISTING 119)	LOC81691 Exonuclease NEF-sp	Homo sapiens exonuclease NEF-sp (LOC81691), mRNA	CCCAGTGACGA CCAAACTCAAA GATGTACAGAG GCAGTTAAAAG CACTGCTTCCT C (SEQUENCE LISTING 48)
49	Hs.194746	NM_018896 (SEQUENCE LISTING 120)	CACNA1G Calcium channel, voltage-dependent, alpha 1G subunit	Homo sapiens calcium channel, voltage-dependent, alpha 1G subunit (CACNA1G), mRNA	ACGTCAGAGAT TGTGTCTGAAC CGTCCTGCTCT CTAGCTCTGAC GGATGA (SEQUENCE LISTING 49)
50	Hs.209061	NM_003831	SUDD SudD	Homo sapiens	TCACGGCCTGG

		(SEQUENCE LISTING 121)	suppressor of bimD6 homolog (A. nidulans)	sudD suppressor of bimD6 homolog (A. nidulans) (SUDD), mRNA	AGTTCTTGTTCC GGGACTGCAGG AATGTCTCGCA GTT (SEQUENCE LISTING 50)
51	Hs.25087	NM_006070 (SEQUENCE LISTING 122)	TFG TRK-fused gene	Homo sapiens TRK-fused gene (TFG), mRNA	TAATCCTTATGC GCGTAACCGTC CTCCCTTTGGT CAGGGCTATAC CCAAC (SEQUENCE LISTING 51)
52	Hs.3017	NM_003284 (SEQUENCE LISTING 123)	TNP1 Transition protein 1 (during histone to protamine replacement)	Homo sapiens transition protein 1 (during histone to protamine replacement) (TNP1), mRNA	GATCAAAGCCA GAGAGGAGCCT ATGGAATGTGG ATCAAATGCCA GTTGTGACG (SEQUENCE LISTING 52)
53	Hs.283664	NM_032466	ASPH	Homo sapiens	GAACCACAACA

		(SEQUENCE LISTING 124)	Aspartate beta-hydroxylase	aspartate beta-hydroxylase (ASPH), transcript variant 3, mRNA	AGAGGATGATG AGTTTCTTATGG CGACTGATGTA GATGATAGATTT GAGACCCTGG (SEQUENCE LISTING 53)
54	Hs.283664	NM_032467 (SEQUENCE LISTING 125)	ASPH Aspartate beta-hydroxylase	Homo sapiens aspartate beta-hydroxylase (ASPH), transcript variant 4, mRNA	CTCAGGGAGAT GGATTTGCTCG TTGTTTTCTTCC CTCCTTCCCCTT CCTG (SEQUENCE LISTING 54)
55	Hs.171992	NM_002843 (SEQUENCE LISTING 126)	PTPRJ Protein tyrosine phosphatase, receptor type, J	Homo sapiens protein tyrosine phosphatase, receptor type, J (PTPRJ), mRNA	CCGTGGATGTG TATGGGATTGT GTATGACCTTC GAATGCATAGG CCTTTAATGGTG C (SEQUENCE

					LISTING 55)
56	Hs.155172	NM_003664 (SEQUENCE LISTING 127)	AP3B1	adaptor-related protein complex 3, beta 1 subunit	GCCCAGCTTAT CATAAACACTGA GAAAACTGTGA TTGGCTCTGTTC TGCTGCGGG (SEQUENCE LISTING 56)
57	Hs.183418	M37712 (SEQUENCE LISTING 128)	CDC2L2	cell dividion cycle2-like2	CGAGAAAATGA AAACCACCTCTT GGTTGTTCCAG AGTCACGGTTC GACCGAG (SEQUENCE LISTING 57)
58	Hs.244473	NM_031900 (SEQUENCE LISTING 129)	AGXT2	alanine-glyoxylate aminotransferase 2	TCCGGGATTGT TACTGTCAGTGT TGGCCATTGCC ACCCAAAGGTG AATGC (SEQUENCE

					LISTING 58)
59	Hs.12835	NM_004842 (SEQUENCE LISTING 130)	AKAP7	A kinase (PRKA) anchor protein 7	GAGCCCGATGA CGCTGAACTAG TAAGGCTCAGT AAGAGGCTGGT GGAGAA (SEQUENCE LISTING 59)
60	Hs.1650	NM_000111 (SEQUENCE LISTING 131)	SLC26A3	solute carrier family 26, member 3	TCAGCCCCCTA TTACACCTGAC GTGGAGACTTT CCAAAACACCG TAGGAG (SEQUENCE LISTING 60)
61	Hs.29981	NM_000112 (SEQUENCE LISTING 132)	SLC26A2	solute carrier family 26 (sulfate transporter), member 2	CAGCAGGGATC CACACACTGAA AGAAGTTCGCA GAGATTATGAA GCCATTGGAAT CC

					(SEQUENCE LISTING 61)
62	Hs.2246	NM_001308 (SEQUENCE LISTING 133)	CPN1	carboxypeptidase N, polypeptide 1, 50kD	TCAAGTAAGCC CTGTGAGGAGA GCTCCCAGCAG AAGGCACGGAG T (SEQUENCE LISTING 62)
63	Hs.267871	NM_005177 (SEQUENCE LISTING 134)	ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	AAATGCTTGATT GCAGAGGTCTG GTGCCCTGTCA CCGACCTTGAC TCCAT (SEQUENCE LISTING 63)
64	Hs.75445	NM_004684 (SEQUENCE LISTING 135)	SPARCL1	SPARC-like 1 (mast9, hevin)	CTGCGAGCATC TCTGGTGCCCA TGGAACACTGC ATAACCCGTTTC TTTGA

					(SEQUENCE LISTING 64)
65	Hs.39957	NM_016445 (SEQUENCE LISTING 136)	PLEK2	pleckstrin 2 (mouse) homolog	TGGCGTTCCCA CTGGGGTTAAA GGGAATGTCCA GGGAAACCTCT TCAAAG (SEQUENCE LISTING 65)
66	Hs.65029	NM_002048 (SEQUENCE LISTING 137)	GAS1	growth arrest-specific 1	CGACTACTACG ATGAGGACTAC GATGACGAGCA GCGCACCGG (SEQUENCE LISTING 66)
67	Hs.239926	NM_006745 (SEQUENCE LISTING 138)	SC4MOL	sterol-C4-methyl oxidase-like	GCTGGTTCTCG GCATCATGATTT CCACCACATGA ACTTCATTGGAA ACTATGCTTCAA C

					(SEQUENCE LISTING 67)
68	Hs.59271	NM_006758 (SEQUENCE LISTING 139)	U2AF1	U2(RNU2) small nuclear RNA auxillary factor 1	TCTGTGACAAC CTGGGAGACCA CCTGGTGGGGA ACGTGTACGTC AAGTTT (SEQUENCE LISTING 68)
69	Hs.8867	NM_001554 (SEQUENCE LISTING 140)	CYR61	cysteine-rich, angiogenic inducer, 61	CAAAACGCAGC CCTGCGACCAC ACCAAGGGGCT GGAATGCAACT T (SEQUENCE LISTING 69)
70	Hs.50123	NM_003452 (SEQUENCE LISTING 141)	ZNF189	zinc finger protein 189	CAACAGCGCAG TCTTGTCAACCA TCAGATGATCC ATGCAGAGGTG AAAACCC

					(SEQUENCE LISTING 70)
71	Hs.82071	NM_006079 (SEQUENCE LISTING 142)	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	CACCAGATGAA CGGGACAAACC AGCACTTCCGA GATTGCAACCC CAAGCA (SEQUENCE LISTING 71)

From the above table, the HS ID of the 71 genes comprises:

Hs.107213 Hs.123107 Hs.1369 Hs.151254 Hs.1526 H s.184270 Hs.2043 Hs.267871 Hs.4935 Hs.5509 Hs.5662 Hs.75990 Hs.83384 Hs.10029 Hs.103982 Hs.12314 Hs.15 0557 Hs.169266 Hs.1827 Hs.1869 Hs.194148 Hs.2352 Hs .246885 Hs.29665 Hs.3235 Hs.55209 Hs.585 Hs.62187 H s.63290 Hs.699 Hs.74111 Hs.75103 Hs.75117 Hs.75236 Hs.75258 Hs.75498 Hs.76913 Hs.79889 Hs.82173 Hs.840 72 Hs.85146 Hs.85844 Hs.88219 Hs.9914 Hs.169319 Hs. 72805 Hs.108301 Hs.177926 Hs.194746 Hs.209061 Hs.25

087 Hs.3017 Hs.283664 Hs.283664 Hs.171992 Hs.155172 Hs.183418 Hs.244473 Hs.12835 Hs.1650 Hs.29981 Hs.2246 Hs.267871 Hs.75445 Hs.39957 Hs.65029 Hs.239926 Hs.59271 Hs.8867 Hs.50123 Hs.82071 etc.

We obtain said specific oligonucleotides sequences by using analysis of OMP (Oligonucleotide Modeling Platform, DNA Software, Inc., Ann Arbor, MI) DNA software, Said gene sequences can act as a reagent, a biochip and a medicine for detecting colorectal cancer shown in table 1.

According to the present invention, FIG. 2a and FIG. 2b are views showing the primary screening. FIG.3a and FIG.3b are views showing affirmation to genes using Northern Blotting method. FIG.4a and 4b are views showing quantity expression of cancer tissue. we search over progressive distinctive new genes among the carcinoma process of colorectal cancer by using SSH method to build up CRA libraries and CRC libraries which make the comparison between adenoma, adenocarcinoma and normal tissue, that obtain over 5000 clones in per library; then randomly select about 3000 clones of cDNA from per library to dot on nylon membrane as pre-screen by using Colony Hybridization shown in FIG.2a and FIG.2b. The high expression colonies in colorectal cancer and adenoma are selected by the Colony

Hybridization and then the nucleic acid of cDNA after purification spot on glass chip by using microarray testing.

The expression profiles of the cDNA chips were derived from a set of cDNA probes including adenoma, adenocarcinoma and the corresponding normal tissue from the same patient. Genes exhibiting at least three-fold greater intensities in the adenocarcinoma or adenoma than in corresponding normal tissue samples were considered significant. The significant up-regulated genes were then further confirmed by Northern blot (FIG. 3a and FIG. 3b) and subsequently sequenced. Northern analysis of each set of cDNA genes on the chip revealed that 36 genes were detected as up-regulated in adenoma compared to normal, and 54 genes were detected as up-regulated in carcinoma as compared to the normal control. A set of 23 genes with serial increase of genes expression from adenoma to carcinoma was identified.

Further, comparison is made by using EMBL/GenBank libraries of NCBI/BLAST program, there are 3 unknown functional genes among 23 identified genes including ectopic viral integration site 2B (Genbank accession no.NM-006495) Homo sapiens chromosome 21q22.1 anonymous mRNA sequence (Genebank accession no.AF003738) and Homo sapiens DMR protein mRNA (Genbank accession no.AF327354), and another 20 functional

genes. Among these 20 functional genes, 6 genes are CRC-related (such as TM4SF3), 14 genes are CRC-unrelated (such as ATP2A2). Moreover, we obtain cDNAs of three patients who suffer from adenoma and adenocarcinama simultaneously and four colorectal cancer patients to affirm variation of 23 identified genes, result shown that were at least 3-fold higher in mRNA expression level in the adenocarcinoma tissues compared with normal samples, and the level gradually increased from colorectal adenomas to adenocarcinomas shown in FIG. 4a and FIG. 4b.

Now, methods of clinical diagnosis for detecting colorectal cancer are fecal occult blood test, image test, tumor label and colonoscopy. In each of these methods, we can generalize purpose of the present invention according to disadvantage of these methods.

1. Early diagnosis

If patient undergo colorectal cancer before tumor cells spread out, five-year survival rate can be achieved over 90%. A certain number of tumor cells are needed for traditional detection by using tumor label method. In the case of image test, normally, correctly affirmation can be made easier when tumor become large. It is high invasion and price to make low acceptance for the patient in the colonoscopy that can not suitable for early diagnosis. Because

of the process of circulating of tumor cells, different expression certainly happen among the genes. In the process of proliferation of early tumor cells, the dying cells cause molecule of ribonucleic acid to release into blood circulation. And, early diagnosis can be offered by the detection of using the constructed oligonucleotide biochip which is discharged from small number of tumor cells in the peripheral blood.

2. Specificity and sensitivity

Fecal occult blood test has shortcomings for high false positives and false negatives to low specificity and sensitivity of the method, therefore the method is merely a first screening tool and the tumor label method is also not high specificity and sensitivity. But, we use these genes to detect peripheral blood of 100 CRC patients, peripheral blood of 50 healthy people and 40 other cancer-related patients as controls shown in FIG.1, these genes can detect 88 colorectal cancer patients for remarkable sensitivity of 88% (88/100) and specificity of 90% (90/100) in the clinical analysis.

3. Safety

The colonoscopy has high invasion and price to make low acceptance for patient in the mass screening tool of early diagnosis. Because sample collection is convenience and low invasion, Peripheral blood test of patient is

a diagnosis method of genes, that is suitable to mass screening clinical application.

Please refer to FIG.5, showing another preferred embodiment according to the present invention. We choose genes of colorectal cancer and vector that express simultaneously in eukaryotic and prokaryotic to form recombination genes, and then form eukaryotic transformant cell by using and further form prokaryotic transfectant cell, and then obtain secreted protein by using extract of genes having said recombination genes, and obtain antibody from said secreted protein immune animals for making of protein testing reagent, colorectal vaccine and colorectal protein medicine for colorectal cancer.

The present invention may be embodied in other specific forms without departing from the spirit of the essential attributes thereof; therefore, the illustrated embodiment should be considered in all respects as illustrative and not restrictive, reference being made to the appended claims rather than to the foregoing description to indicate the scope of the invention.

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